Table 4. Survival of Rat Ovarian Tissue grafted into Pyridoxine-deficient Mice

Preparatory treatment for 4 weeks	Strain of mouse	Proportion of animals developing active grafts	Proportion of animals with active grafts at 28 days
None	*PA*	11/40	0/40
2·5 mgm. deoxypyridoxine per day	*PA*	10/37	6/37
None	*PDE*	9/19	0/19
2·5 mgm. deoxypridoxine per day	*PDE*	28/40	12/40

Striking results were obtained with heterografts of rat ovaries made into treated mice (Table 4).

In the third group of mice the surviving grafts were removed by operation after five weeks and were found to be relatively very large and, although heavily infiltrated with leucocytes, were found on histological examination to possess numerous follicles and corpora lutea. In general, these heterografts were very different from the few small degenerate specimens which can be found in untreated mice at five weeks after the subcutaneous implantation of rat ovarian tissue.

The effect of pyridoxine deficiency in depressing the reaction against ovarian homografts is greater than that of a single dose of whole-body irradiation in mice[5] and of the same order as that which can be obtained in rats by pre-conditioning the prospective recipient with suspensions of homologous tissue[6]. It is less permanent according to present experience than the effect which can be obtained by the induction of tolerance early in life[7].

While these experiments were in progress, Axelrod and his collaborators[8], in continuation of their earlier work, published the results of experiments on intra-strain skin grafting in pyridoxine-deficient rats. In two strains of rats they found that the percentage of takes in deficient rats was far higher than in normal ones, and in one strain survival at 10 weeks was also far better. Experiments on man, however, failed to give analogous results, possibly because the degree of pyridoxine-deficiency was inadequate[9].

So far as rats and mice are concerned, however, it seems clear that the reaction against skin and ovarian homografts respectively is much reduced by pyridoxine deficiency. According to Axelrod *et al.*[8], "it is possible that this effect is related to an inhibition of the immune response to the antigens of the donor skin in this deficiency state". With this suggestion, it seems desirable to postpone discussion until more facts are available.

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DIAPHRAGMS FOR ANALYSING THE DEVELOPMENT OF CONNECTIVE TISSUE

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To gain fundamental information about the nature of any complex biological structure or process, we have to develop techniques which permit us to analyse it by breaking it down into its constituent elementary units; the dissection of organs, the preparation of pure microbial or tissue cultures are examples in point. The study of the laws that govern the developmental mechanics of connective tissue (for example, during normal growth, wound healing and inflammation) has been greatly handicapped by the lack of techniques suitable for an analysis of this kind. Normally, the cells and intercellular structures of connective tissue form an extremely complex maze. Even the simplest linear wound elicits a multitude of interdependent regenerative processes the elements of which (exudate, fibrin, fibres, cells, vessels) interlace in all directions, so that a study even remotely resembling an 'elementary analysis' of the constituents becomes virtually impossible. Accordingly, the scope of the conventional techniques for the study of connective tissue is necessarily limited to the description of histological or chemical components, the uptake of vital dyes, the speed of cicatrization, the measurement of tensile strength, etc.

The object of this communication is to describe the design of diaphragms which, when introduced into connective tissue, present it with simple problems of organization, in that they block growth except along one, or more, regular, predetermined channels. Thus we can obtain single beams of connective tissue, or cords intersecting at any desired angle; these, like the parallel beams of light produced by optical diaphragms, lend themselves to reveal the underlying order in an otherwise confusing complexity.

All experiments were performed on female Sprague–Dawley rats, with an initial body-weight of 100–150 gm. The sterilized diaphragms were introduced beneath the shaved dorsal skin, under ether anæsthesia. Groups of rats were then killed at weekly intervals, to examine the progress of organization macro- and microscopically.

Analyser diaphragms may be prepared of various materials (plastics, china, rubber, paraffin-coated paper, etc.), but glass is the most satisfactory: it can easily be shaped, chemically cleaned and sterilized, its hard, smooth surface prevents adhesion of tissue to the wall, and its transparency facilitates examination of the contents.

Fig. 1 illustrates a few of the basic patterns of tissue-diaphragms that were employed for analysis.

The simple tube, open at both ends (Fig. 1*A*, *B*, *C*), permits only unidirectional growth, in the form of a

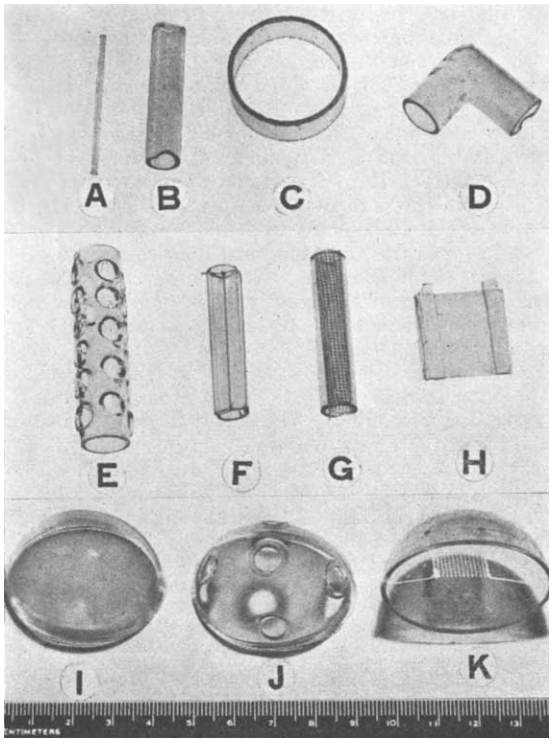

Fig. 1. Samples of various tissue diaphragms

connective-tissue cord that connects the two orifices. The length and calibre of the tube vary in accordance with the problems to be studied; capillaries and small-bore tubes have the additional advantage of being readily implantable, not only into loose connective tissue, but also into muscles, pleura, peritoneum and various parenchymatous organs. Tubes with one end closed revealed that a minimum of two 'tissue bridgeheads' is necessary for rapid organization of a gap, while L-shaped (Fig. 1*D*) and branching tubes (in the form of a Y, T or +) are employed to examine the formation of anastomosing tissue cords at predetermined angles. One perforation or more in the wall of the tube (Fig. 1*E*) offers additional possibilities for the establishment of tissue connexions within a single cylinder (that is, unguided by restraining individual tubular walls around each tissue-cord). Simple, straight tubes, open at both ends, containing a central nylon thread (Fig. 1*F*), an inner parietal lining of fine nylon trellis (Fig. 1*G*), or a central flat 'ladder' of nylon trellis that connects the two open ends revealed that this additional internal scaffolding is especially effective in accelerating organization along the threads. Organization is also expedited by enveloping the diaphragms in nylon trellis; the rapid invasion of the latter by connective tissue furnishes a stabilizing capsule for the whole object. Double plates (two coverglasses, their surfaces slightly separated by lateral glass ledges) offer the possibility of studying two-dimensional invasion of a flat space (Fig. 1*H*); the tissue formed in this can then be examined by merely placing the entire object under the microscope. Hemispheres, or—because of the shape of the rat's back—hemi-ellipsoids are used to examine factors which influence the three-dimensional organization of rounded cavities. These structures offer no entry to tissue, except at their base (Fig. 1*I*), or have one additional opening or more on the roof (Fig. 1*J*) for the exploration of simple or complex tissue-bridge formation. Finally, minute perforations in the roof (which can afterwards be sealed with a drop of paraffin) make it possible to attach threads or trellises of nylon at any point of the roof, thus connecting it with the base (Fig. 1*K*). It is then possible to explore the effectiveness of the nylon scaffolds as tissue guides.

Fig. 2 shows a hemispherical analysing diaphragm with multiple perforations, under the shaved dorsal skin of a rat, two weeks after implantation. The scar of the incision, through which the object had been introduced (dark spots near the root of the tail), is almost healed; it did not interfere with organization within the diaphragm, because the latter was pushed forward at some distance from the wound to prevent the development of external adhesions and diminish the probability of infection. Owing to the absorption of air from the cavity of the hemisphere, the skin over the perforations is slightly depressed. The depth of these depressions remains limited, however, because, soon after implantation, exudation begins and a characteristic, citrine, thick fluid fills out the empty space. This fluid does not coagulate *in situ*, but has some tendency to do so after evacuation. Eventually, fibrin threads, covered and invaded by histiocytes, connect the parietal openings with the base and thus provide temporary bridges. These are later organized by connective tissue and invaded by blood vessels.

This organization is most clearly illustrated by the simple, unidirectional growth that occurs in straight glass tubes (Fig. 3).

Two weeks after implantation of such a tube (3 cm. long, 0·8 mm. wide, and enveloped in a nylon trellis for better fixation), a straight axial cord has developed between the two openings (Fig. 3*A*). This cord consists of a broad, central spindle made up of fibrin threads and histiocytes. It is attached to the centre of the end-plates by a narrow filament of connective tissue, Van Gieson-positive and well vascularized. By this time, such tubes are completely or almost completely filled with citrine fluid

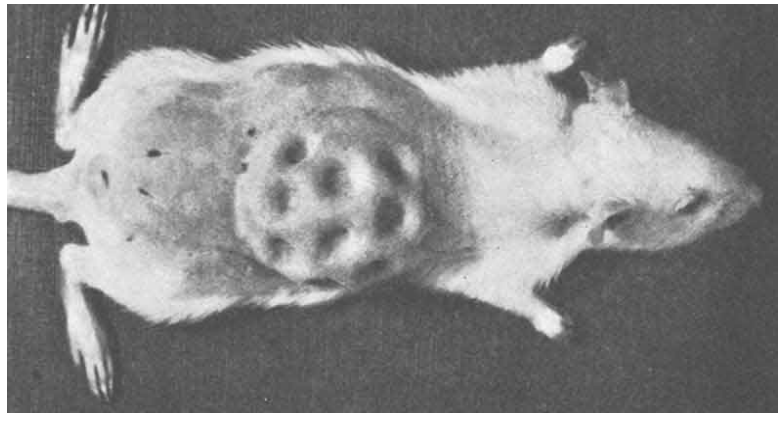

Fig. 2. Hemispherical tissue diaphragm as it appears *in situ*

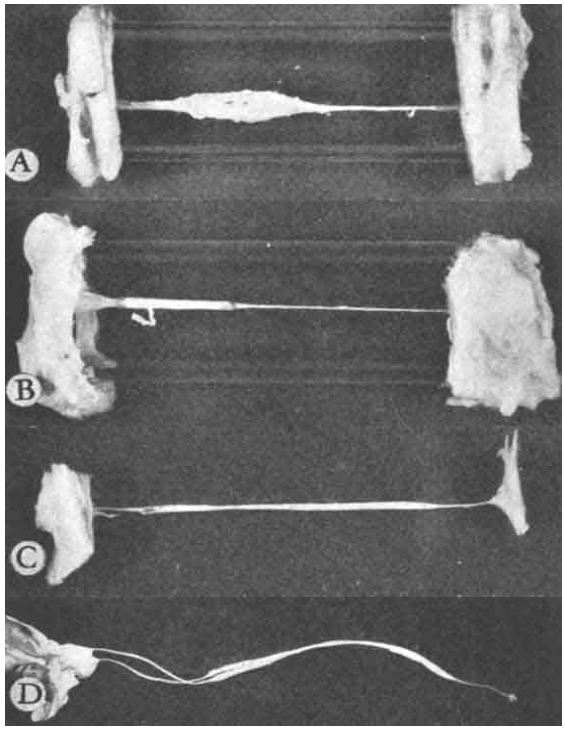

Fig. 3. Various stages and types of axial-cord formation within simple glass tubes. In *A* and *B* the tube and the disk-like connective-tissue plates at the opening are intact; in *C* and *D* the contents are represented after removal from the tube

and, although the two end-plates of connective tissue are broad, the bridge is invariably narrow and attached centrally.

After one month, the fibrin spindle becomes narrower and shorter (Fig. 3*B*); the 'growth line' that separates the light fibrinous centre from the darker invading tissue is reddish in the living animal. Quite commonly, invasion by connective tissue progresses much more rapidly on one side (in Fig. 3*B* on the right) than on the other, but the development of fibrin threads between the spindle and the glass wall (which apparently occurred here because of a rough dirt speck on the wall) is exceptional. It is also quite uncommon to find an accessory, abortive, second cord, attached unilaterally (Fig. 3*C*), or a complete Y-shaped, split cord, in simple straight tubes (Fig. 3*D*). Curiously, within the limits which have been studied so far, the speed with which the two openings are connected by an axial cord, and the thickness of these bridges, are largely independent of the length and width of the glass tubes.

The details of these investigations will be described elsewhere. Here, attention is directed merely to the possibility of analysing the mechanics of tissue growth by the selective restriction of proliferation to certain channels. It is hoped that such analysing diaphragms may prove useful in the study of developmental mechanisms under normal conditions and after the topical or systemic application of growth-modifying stimuli.

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ESTABLISHMENT OF PARENCHYMATOUS SPLENO-PULMONARY VENOUS ANASTOMOSIS

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A SUCCESSFUL shunt between the splenic vein and the pulmonary venous system via a parenchymatous anastomosis in the dog has been achieved. The investigation was conducted in an attempt to find a solution to the problem of extrahepatic obstruction of the portal venous system in young children. At present, this condition in children is not as amenable to successful treatment by current methods of shunt operation as in adults. Further work will be necessary before application of this experimental work to clinical problems can be undertaken.

Dogs 8–20 kgm. in weight were used. General anæsthesia was induced and continued with thiopentone and succinylcholine chloride in divided doses, respiration being maintained by manual control of flow of oxygen through an endotracheal tube. Tetracycline was administered by mouth for ten days, beginning the day before operation, to avoid the high incidence of acute empyema which occurred post-operatively in the early attempts.

A thoraco-abdominal incision was made in the line of the eighth rib on the left side, and the spleen delivered through the wound. A bulldog clamp was temporarily applied to the splenic pedicle. Most of the spleen was removed, leaving part of the lower pole, measuring approximately 3 × 2 × 1 cm. at the site of entry of the main lower division of the splenic vessels. The splenic capsule was removed except that on the hilar surface.

Next, a Satinsky vascular clamp was placed across the lower part of the lower lobe of the left lung, to act both as a retractor and as a hæmostat. An incision about 3 cm. long was made in the lung on either the costal or diaphragmatic surface, and continued deep into its substance. After bringing the splenic pedicle up through the tendinous part of the diaphragm, the remnant of the spleen was inserted into the opening in the lung and the lung sewn over it.

All clamps were removed and an intercostal drain inserted and connected to an underwater seal. The wound was closed in layers.

The operation is relatively easy to perform, and the procedure is usually completed in 1–2 hr. The overall operative and post-operative mortality was